

# Evaluating *Tripsacum*-introgressed maize germplasm after infestation with western corn rootworms (Coleoptera: Chrysomelidae)

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*Diabrotica*, gamagrass, host plant resistance, maize breeding, root pests

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## Abstract

Maize (*Zea mays* L.) is a valuable commodity throughout the world, but corn rootworms (Chrysomelidae: *Diabrotica* spp.) often cause economic damage and increase production costs. Current rootworm management strategies have limitations, and in order to create viable management alternatives, researchers have been developing novel maize lines using Eastern gamagrass (*Tripsacum dactyloides* L.) germplasm, a wild relative of maize that is resistant to rootworms. Ten maize *Tripsacum*-introgressed inbred lines derived from recurrent selection of crosses with gamagrass and teosinte (*Zea diploperennis* Iltis) recombinants and two public inbred lines were assessed for susceptibility to western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and yield in a two-year field study. Two experimental maize inbred lines, SDG11 and SDG20, had mean root damage ratings that were significantly lower than the susceptible public line B73. Two other experimental maize inbred lines, SDG12 and SDG6, appeared tolerant to rootworm damage because they exhibited yield increases after rootworm infestation in both years. In the majority of cases, mean yield per plant of experimental maize lines used in yield analyses was equal to or exceeded that of the public inbred lines B73 and W64A. Our study indicates that there is potential to use *Tripsacum*-introgressed maize germplasm in breeding programs to enhance plant resistance and/or tolerance to corn rootworms, although further research on insect resistance and agronomic potential of this germplasm needs to be conducted in F<sub>1</sub> hybrids.

## Introduction

Corn rootworm larvae consume maize root tissues, thus negatively impacting plant physiology and function (Riedell 1990; Hou et al. 1997; Riedell and Reese 1999), and impeding harvesting due to stalk lodging (Sutter et al. 1990). Ultimately, larval rootworm feeding reduces yield (Sutter et al. 1990; Spike and Tollefson 1991), and can cause significant economic losses (Metcalf 1986).

Pesticides, crop rotation, and transgenic hybrids that express insecticidal proteins derived from

*Bacillus thuringiensis* Berliner (Bt) are commonly used to manage larval rootworm populations (Wilson et al. 2005), although there are drawbacks and limitations to current control methods. Broad-spectrum pesticides applied as seed treatments, directly to the soil, and for adult rootworm control can negatively affect human health and the environment, and corn rootworms have evolved resistance to some of these insecticides (Ball and Weekman 1962; Meinke et al. 1998). In some areas, rootworms have adapted to crop rotation by laying eggs in alternate crops such as soybean, *Glycine max* L. (Levine et al. 2002;

Rondon and Gray 2004), or extending their diapause (Krysan et al. 1984; Levine et al. 1992). Recently registered transgenic Bt hybrids target corn rootworms. However, their utility would be severely compromised if pests evolved resistance. Due to non-selective pesticide use, decline of crop rotation effectiveness, and the presence of corn rootworms in Europe (Kiss et al. 2005), there is an increasing need for alternate rootworm management strategies. Furthermore, non-transgenic hybrids are desirable for use in certain situations, including organic fields, refuges required for insect resistance management, and when producers are hesitant or unable to use genetically modified organisms.

Plant breeders and entomologists have been searching for rootworm resistant and/or tolerant maize lines for decades (Branson et al. 1983; Gray and Steffey 1998; Hibbard et al. 1999). Research has focused on using germplasm of maize and its relatives for use in breeding programs (Branson 1971; Moellenbeck et al. 1995; Hibbard et al. 1999; Eubanks 2002, 2006; Prischmann et al. 2007), refining how plant susceptibility is evaluated (Moellenbeck et al. 1994; Knutson et al. 1999), and investigating resistance mechanisms (Ajani and Lonnquist 1979; Xie et al. 1992; Assabgui et al. 1995).

Several plant species can contribute to corn rootworm development and survival in the field, including grassy weeds and grass species (Branson and Ortman 1970; Clark and Hibbard 2004; Oyediran et al. 2004; Wilson and Hibbard 2004). In contrast, Eastern gamagrass (*Tripsacum dactyloides* L.) is a wild relative of maize that is resistant to corn rootworms via non-preference and/or antibiosis (Branson 1971; Moellenbeck et al. 1995; Eubanks 2001). However, maize-*Tripsacum* hybrids are usually sterile and thus cannot pass on genes responsible for rootworm resistance (Eubanks 1997). Eubanks (1997, 2001, 2002) crossed *Tripsacum* with diploid perennial teosinte (*Zea diploperennis* Iltis, Doebley and Guzmán), another maize relative, and produced viable recombinants that were cross-fertile with maize. This allowed the incorporation of *Tripsacum* genetic material into corn and development of experimental lines, some of which exhibited rootworm resistance, as evidenced in insect bioassays and field root damage ratings (Eubanks 2002, 2006). Continued breeding has produced new *Tripsacum*-introgressed maize inbred lines that need to be evaluated for rootworm susceptibility and agronomic performance under field conditions.

Our objectives were to evaluate the susceptibility to western corn rootworms (Chrysomelidae:

*Diabrotica virgifera virgifera* LeConte) and agronomic performance of 10 experimental inbred lines derived from *Tripsacum*-introgressed maize germplasm and two susceptible public inbred lines in the field after infestation with rootworm eggs. We used the previously established Iowa 1–6 root damage rating scale (Hills and Peters 1971; Branson 1986; Mayo 1986) to assess root damage.

## Materials and Methods

### Experimental plot setup

The experiment was conducted in 2005 and 2006 at the Eastern South Dakota Soil and Water Conservation Research Farm near Brookings, SD (USDA, Agricultural Research Service, Northern Plains Area). Corn lines were planted in fields managed under a four-year rotation of corn, soybeans, oats, and spring wheat. Crop rotation was used to ensure that experimental plots were not contaminated by surrounding natural rootworm populations, which would increase variability within the study.

In order to determine appropriate fertilization levels, the soil was sampled on 4 April 2005 and 12 April 2006 from five locations throughout the field. At each location, five samples were taken from two depths (0–15 and 15–61 cm), and sent to a soil testing lab (South Dakota State University, Soil Testing Lab, Brookings, SD). On 29 April 2005, 157 kg/ha starter fertilizer (14-36-13) and 151 kg/ha urea (46-0-0) were surface applied, while on 4 May 2006, 177 kg/ha starter (14-36-13) and 105 kg/ha urea (46-0-0) were applied. Fertilizer was added to the field (19 m × 20 m) according to soil testing recommendations for 7500 kg/ha yield, and incorporated into the top 10.2 cm of soil via field cultivation.

There were 12 maize lines used in the study: 10 experimental inbred lines (SDG6, SDG7, SDG9, SDG10, SDG11, SDG12, SDG15, SDG17, SDG19, and SDG20) and two susceptible publicly available inbred lines (B73 and W64A). None of the experimental or public inbred lines had seed treatments. The original crosses made to establish experimental populations (Eubanks 1997, 2001, 2002) involved *Tripsacum dactyloides*, which is resistant to corn rootworms, crossed with *Zea diploperennis*, resulting in fertile intergeneric hybrids. All experimental lines were introgressed with genes from one or two *Tripsacum-diploperennis* recombinant lines (Tripsacorn and/or Sun Star) (Eubanks 2002, 2006). Tripsacorn has a tetraploid *T. dactyloides* as the seed parent (Source: Indiana University, Bloomington, IN; originally

collected from Santa Claus, Spencer County, IN; 1949–1954) and *Z. diploperennis* as the pollen parent (Source: Upper las Joyas, Sierra de Manantlan, Jalisco, Mexico; Iltis, Nee & Guzman Acc. #1250; 1979). In contrast, Sun Star has *Z. diploperennis* as the seed parent (Source: Jalisco, Mexico; R. Guzman M. Acc. #777) and a diploid *T. dactyloides* as the pollen parent (Source: Manhattan, KS; K. Anderson). SDG6, SDG9, SDG15, and SDG19 have *T. dactyloides* cytoplasmic genes, meaning that *T. dactyloides* was the female parent of the bridging cross with *Z. diploperennis*. For SDG7, SDG10, SDG11, SDG12, and SDG17, *Z. diploperennis* was the female parent in the cross. SDG20 was derived from a three-way cross with parentage tracing to *T. dactyloides* as the female parent for two individuals and to *Z. diploperennis* as the female parent of the third. Reciprocal crosses were then performed between maize and the intergeneric hybrids (*T. dactyloides* × *Z. diploperennis* or *Z. diploperennis* × *T. dactyloides*), and the resulting trigeneric hybrid plants backcrossed to maize or one of the intergeneric hybrids. The experimental inbred lines used in this study were derived from a recurrent selection breeding program using the maize inbreds B73 and W64A with a minimum of 14 generations of recurrent selection, backcrossing, and selfing. For more detailed information on recurrent selection methods, development of the experimental inbred lines, and genes involved in expression of rootworm tolerance see Eubanks (1998, 2002, 2003, 2006). The Sun Dance Genetics (SDG) lines in this study ranged from approximately 72% corn 28% exotic, to 97% corn 3% exotic.

The study consisted of two experiments conducted simultaneously: an evaluation of maize line susceptibility to larval corn rootworm feeding damage (root damage ratings, root fresh weight), and an evaluation of how rootworm infestation impacted grain yield. Experiments were conducted in adjacent blocks within the same field. We utilized randomized complete block designs with four replicates per experiment. Experimental maize inbred lines were planted within east-west single-row experimental plots with 0.76 m row spacing. Each experimental row was separated by a buffer row.

On 16 May 2005, buffer rows (DeKalb® 440) were sown at the label recommended density of 74 130 seeds/ha using an eight row vacuum planter (Max Merge 7200, John Deere, Moline, IL), while on 12 May 2006 buffer rows (DeKalb® 46-26) were sown at the label recommended density of 63 010 seeds/ha. Hybrid lines were used in buffer rows to protect inbred lines from adverse weather

conditions, especially wind damage. While hybrid maize plants were approximately 2.0–2.5 m high and may have shaded some inbred maize lines, these effects were uniform across experimental rows. Maize inbred lines were hand-planted on 19 May 2005 and 16 May 2006 using jab planters (Easy-Plant Model 98; R.T. Adkins, Parsonsburg, MD) with eight seeds of one maize line per single-row plot, 5 cm seed depth, and 23 cm plant spacing. There were no formal buffer plants between experimental plots planted in the same row, however, the first and last plant in each experimental plot were not sampled. There was 0.76 m of buffer plants (2005, DeKalb® 440, 94 days relative maturity; 2006, DeKalb® 46-26, 96 days relative maturity) on the end of each row of experimental maize plants.

For evaluating maize line susceptibility to rootworms, all rows with experimental maize inbred lines were infested with rootworm eggs. In contrast, the second experiment evaluating grain yield was a split plot experiment with two treatments, (1) an agar-only control and (2) rootworm infested plots. Rootworm-treated rows were mechanically infested on 17 May 2005 and 15 May 2006 with 1000 viable western corn rootworm eggs per 30 cm suspended in a 0.15% agar solution (Palmer et al. 1977) at an approximate depth of 10 cm using Sutter and Branson's (1980). Control plots in the second experiment were mechanically infested with only the 0.15% agar solution. Buffer plants were not infested with rootworm eggs.

Rootworm eggs were obtained from the primary diapausing colony maintained at the North Central Agricultural Research Laboratory in Brookings, SD. Hatch controls were performed prior to infestation to determine the percentage of viable eggs. Using a fine paintbrush, three batches of 100 eggs were placed on moistened filter paper in separate Petri dishes (100 mm × 15 mm), incubated at 25°C, and monitored for up to 4 weeks. In 2005, 87% ± 2% of the eggs hatched, while in 2006, 86% ± 4% of the eggs were viable.

In order to estimate western corn rootworm larval development and maximum root feeding damage, on 18 May 2005 and 16 May 2006 we placed the soil probe of a biophenometer (Model: BIO-51-TP03C; Omnidata® datapod, Logan, UT) to a depth of 10 cm into the soil and monitored soil temperature and growing degree days (GDD) with an upper threshold of 35°C and a lower threshold of 11°C (Fisher et al. 1990). Corn rootworm development is linked to temperature (Jackson and Elliott 1988; Woodson and Jackson 1996), and maximum

rootworm damage occurs around the time adults begin emerging (Branson 1986).

### Root damage and root fresh weight

On 13 July 2005 and 12 July 2006, four root systems per plot were sampled and rated for root damage. Root systems were removed from the soil when the majority of the insect population reached the pupal stage of development, which occurs approximately 600<sub>(base 11)</sub> GDD after egg infestation (Riedell and Evenson 1993). Plant shoots were cut above the lowest visible node and discarded, and the remaining stalk was labelled with a water proof (Tyvek) tag attached with a cable tie. The root systems were dug with a four pronged potato fork. Loose soil was removed from the root systems in the field by gentle tapping. Root systems were then soaked outside in mesh baskets suspended in tanks of water with water softener (1.9 l/tank; Calgon®, Reckitt Benckiser Inc., Wayne, NJ) to help disperse soil aggregates. In 2005, roots were soaked for 1–5 days, while in 2006, roots were only soaked for 1 day. After soaking, root systems were laid across wire mesh baskets and gently washed with high pressure sprayers to remove remaining soil without damaging the roots. Although some deterioration occurred while roots were soaking, it was primarily confined to the stem and did not interfere with root washing and processing. Root systems were then placed within doubled plastic garbage bags and stored in a 7°C cold room to retain moisture and prevent deterioration.

After 6–8 days in cold storage, shoots were cut at the seventh node, the top portion of the stem was discarded, and root system fresh weight was recorded. Root systems were then rated for rootworm larval feeding damage using the Iowa 1–6 scale (Hills and Peters 1971). This rating scale is based upon the following criteria: 1 = no root damage or a few feeding scars, 2 = feeding scars, but no roots pruned to 3.8 cm of the plant, 3 = several roots pruned to 3.81 cm, but an entire node of roots not pruned, 4 = one node of roots pruned, 5 = two nodes pruned, 6 = three or more nodes pruned.

### Maize agronomics

To assess the impact of larval feeding on grain yield of the experimental maize lines, all ears were hand harvested from both non-infested control plots and rootworm infested plots from the second experiment. From 17 October 2005 to 21 October 2005

and from 23 October 2006 to 25 October 2006, ears were picked from all plants within the plots, excluding the first and last plant in each plot, for a maximum of six sampled plants. Ear damage from insect pests was negligible. Ears were placed in tightly-woven mesh bags, labelled, and air dried at approximately 21–26°C in a greenhouse for 7–10 days. Grain was removed from the cobs by feeding ears through a modified corn sheller (McCormick-Deering; International Harvester Co., Chicago, IL), and any missed kernels removed by hand. Grain was then cleaned using an Almaco grain cleaner (Allan Machine Co., Nevada, IA), which blows air over the sample, thus removing chaff and other light debris. The cleaned grain was weighed on a Mettler PC 4400 balance (Mettler Instrument Corp., Hightstown, NJ) and tested for percent moisture using a John Deere Moisture Chek Plus™ (John Deere, Moline, IL). These data were used to extrapolate grain yield at a 150 g/kg moisture basis.

Plant and primary ear node height were measured on three plants per plot in three replicates. Plant height was measured from the ground to the tip of the tassel using a digital reading measuring pole (Sokkia, Senshin Industry Co., Ltd, Osaka, Japan), while primary ear node height was measured from the ground to the uppermost ear node on the stem.

### Data analysis

Root fresh weight, grain yield, plant height, and primary ear node height data were  $\log(X + 1)$  transformed prior to analysis. All data were analysed using the GLIMMIX procedure (SAS® 2004, 2005) followed by Tukey's Honest Significant Difference multiple comparison test (Ramsey and Schafer 1997). Bartlett's test was used to assess the homogeneity of error variances. Because pollen availability can influence yield (Uribe-larrea et al. 2002; Westgate et al. 2003), in yield analyses average days to anthesis was used as a covariate. Average days to anthesis values were calculated from 2 to 5 years of data collected from nurseries in North Carolina and Florida. Some experimental inbred lines had poor germination and ear development, thus, if plants did not germinate, yield data were reported as missing values. If plants germinated but did not produce ears with grain, yield was considered zero. Four lines had extremely poor germination and/or ear development (SDG10, SDG11, SDG15, SDG19), and while mean yield and days to anthesis data were reported, these lines were excluded from statistical analyses of yield data.



## Results

### Root damage ratings

Year did not significantly influence root damage ratings (Year,  $df_{1,65}$ ,  $F = 2.81$ ,  $P = 0.10$ ; Year  $\times$  Line,  $df_{11,65}$ ,  $F = 1.65$ ,  $P = 0.11$ ), and error variances between years were homogeneous ( $P = 0.42$ ), thus data from 2005 and 2006 were used in a combined analysis. Maize line had a significant impact on root damage ratings ( $df_{11,77}$ ,  $F = 4.64$ ,  $P < 0.001$ ). Maize lines SDG11 and SDG20 had the lowest mean root damage ratings, which were significantly lower than the susceptible, publicly available inbred B73, but not W64A (table 1). Maize lines SDG7 and SDG9 had the highest mean root damage ratings, but were only significantly different from SDG11 and SDG20.

### Root fresh weight

Maize root system fresh weight was significantly lower in 2005 than 2006 (Year,  $df_{1,65}$ ,  $F = 313.06$ ,  $P < 0.0001$ ), which may be related to an overabundance of moisture during the early growing season. However, the interaction between year and maize line was not significant (Year  $\times$  Line,  $df_{11,65}$ ,

**Table 1** Mean root damage ratings ( $\pm$  SEM; Hills and Peters 1971) and root fresh weight for 10 experimental maize inbred lines and two public lines

Maize line <sup>1</sup>	RDR <sup>2</sup>	Fresh wt. (g) <sup>3</sup>
	2005 + 2006	2005 + 2006
SDG11	2.4 $\pm$ 0.2 a	46.9 $\pm$ 6.5 a
SDG20	2.5 $\pm$ 0.2 a	48.6 $\pm$ 6.7 a
SDG10	3.0 $\pm$ 0.4 ab	55.5 $\pm$ 9.5 a
SDG6	3.1 $\pm$ 0.2 abc	45.1 $\pm$ 7.5 a
SDG12	3.5 $\pm$ 0.3 abc	36.2 $\pm$ 5.8 a
W64A	3.5 $\pm$ 0.4 abc	36.1 $\pm$ 5.7 a
SDG19	3.7 $\pm$ 0.3 abc	30.4 $\pm$ 6.1 a
SDG17	3.7 $\pm$ 0.3 abc	37.0 $\pm$ 5.9 a
SDG15	3.7 $\pm$ 0.6 abc	32.3 $\pm$ 5.8 a
B73	4.0 $\pm$ 0.3 bc	47.4 $\pm$ 7.6 a
SDG9	4.2 $\pm$ 0.3 bc	41.9 $\pm$ 6.0 a
SDG7	4.5 $\pm$ 0.1 c	38.0 $\pm$ 6.5 a
All lines	3.5 $\pm$ 0.1	41.3 $\pm$ 1.9

Within each column means  $\pm$  standard error of the mean followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>1</sup>W64A and B73 are susceptible public inbreds.

<sup>2</sup>RDR = root damage ratings using the Iowa 1–6 scale (1 = no root damage or a few feeding scars, 2 = feeding scars, but no roots pruned to 3.8 cm of the plant, 3 = several roots pruned to 3.8 cm, but an entire node of roots not pruned, 4 = one node of roots pruned, 5 = two nodes pruned, 6 = three or more nodes pruned).

<sup>3</sup>Fresh roots were cut at the seventh node and weighed.

$F = 1.23$ ,  $P = 0.29$ ), and error variances between years were homogeneous ( $P = 0.19$ ), thus data from 2005 and 2006 were combined for analysis. While inbred lines SDG15 and SDG19 had the lowest mean root fresh weight, and SDG10 and SDG20 had the highest mean root fresh weight, maize line did not significantly impact root fresh weight ( $df_{11,77}$ ,  $F = 1.22$ ,  $P = 0.29$ ; table 1).

### Maize yield

The majority of experimental inbreds had germination rates that were comparable to the two public inbreds, B73 and W64A (table 2). However, three experimental inbreds (SDG11, SDG10, SDG15) often had germination rates below 50%. In addition, when

**Table 2** Per cent germination and ear development of maize lines

Maize line <sup>1</sup>	Infestation status <sup>2</sup>	% Plants germinated <sup>3</sup>		% Germinated plants with no ear development <sup>4</sup>	
		2005	2006	2005	2006
SDG11	C	42	25	0	0
	WCR	54	8	0	50
SDG20	C	75	67	0	0
	WCR	92	75	27	44
SDG10	C	13	42	0	0
	WCR	54	13	0	33
SDG6	C	54	88	0	0
	WCR	75	75	0	0
SDG12	C	67	75	0	0
	WCR	75	71	0	0
W64A	C	92	96	23	0
	WCR	88	71	0	0
SDG19	C	67	96	69	0
	WCR	92	88	77	100
SDG17	C	75	67	0	0
	WCR	71	75	0	0
SDG15	C	38	67	67	25
	WCR	33	75	100	44
B73	C	58	54	0	39
	WCR	83	88	0	24
SDG9	C	83	50	20	0
	WCR	83	75	0	0
SDG7	C	63	83	0	0
	WCR	88	75	0	0

<sup>1</sup>W64A and B73 are susceptible public inbreds.

<sup>2</sup>C = non-infested control plants, WCR = plants infested with western corn rootworm eggs.

<sup>3</sup>Per cent germination was calculated from the six plants used for yield; the first and last plants in each plot were buffer plants and were not used to assess yield.

<sup>4</sup>Ears were considered as non-developed if there was an ear present, but it had no grain kernels.

**Table 3** Mean yield ( $\pm$  SEM) per plant for 10 experimental maize inbred line and two public lines

Maize line <sup>1</sup>	DTA <sup>2</sup>	Yield/plant (g) <sup>3</sup> 2005			Yield/plant (g) <sup>3</sup> 2006		
		C	WCR	% diff	C	WCR	% diff
SDG11*	56	62.4 $\pm$ 9.3	55.7 $\pm$ 8.0	-11	120.2 $\pm$ 11.3	57.1 $\pm$ 57.1	-53
SDG20	59	31.4 $\pm$ 3.5 a	18.9 $\pm$ 6.4 a	-40	55.5 $\pm$ 10.1 a	14.4 $\pm$ 11.8 b	-74
SDG10*	65	18.5 $\pm$ 4	26.5 $\pm$ 4.6	+43	102.6 $\pm$ 24.9	66.1 $\pm$ 66.1	-36
SDG6	65	22.4 $\pm$ 5.7 a	29.4 $\pm$ 5.4 a	+31	53.6 $\pm$ 12.1 a	74.6 $\pm$ 19.7 a	+39
SDG12	69	35.8 $\pm$ 11.2 a	52.9 $\pm$ 9.8 a	+48	60.6 $\pm$ 12.3 a	70.4 $\pm$ 27.9 a	+16
W64A	63	20.5 $\pm$ 7.4 a	18.7 $\pm$ 4.3 a	-9	73.6 $\pm$ 8.1 a	66.1 $\pm$ 12.1 a	-10
SDG19*	70	4.8 $\pm$ 4.8	0.9 $\pm$ 0.9 <sup>5</sup>	-81	18.1 $\pm$ 3.2	0 $\pm$ 0	-100
SDG17	56	70.2 $\pm$ 4.5 a	51.3 $\pm$ 7.5 a	-27	47.8 $\pm$ 9.0 a	44.7 $\pm$ 17.3 ab	-7
SDG15*	74	5.7 $\pm$ 5.7	0 $\pm$ 0	-100	16.1 $\pm$ 10.1	9.1 $\pm$ 5.7	-44
B73	63	54.1 $\pm$ 8.0 a	28.4 $\pm$ 7.6 a	-48	34.2 $\pm$ 18.1 a	21.5 $\pm$ 9.9 ab	-37
SDG9	59	40.8 $\pm$ 13.7 a	52.5 $\pm$ 8.5 a	+29	67.0 $\pm$ 10.4 a	40.0 $\pm$ 18.3 ab	-40
SDG7	63	61.2 $\pm$ 4.6 a	46.5 $\pm$ 7.1 a	-24	66.0 $\pm$ 10.1 a	49.7 $\pm$ 9.3 a	-25
All lines		44.6 $\pm$ 3.7	38.2 $\pm$ 3.0	-14	61.5 $\pm$ 5.2	54.8 $\pm$ 6.6	-11

Within each column means  $\pm$  standard error of the mean followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>1</sup>W64A and B73 are susceptible public inbreds.

\* = maize lines not included in statistical analyses due to poor germination and/or ear development.

<sup>2</sup>DTA = average days to anthesis based on data from North Carolina and Florida nurseries collected over 2–5 years.

<sup>3</sup>C = non-infested control plants; WCR = plants infested with western corn rootworm eggs; % diff = difference in yield between non-infested and infested plants.

<sup>4</sup>No standard error because plants germinated and produced ears in only 1 replicate (plants in other replicates did not germinate, thus data were considered missing).

<sup>5</sup>Standard error equal to the mean because plants only produced ears in 1 replicate (plants in other replicates germinated, but did not produce ears, thus data were considered zero).

SDG15 and SDG19 germinated, plants often had poor ear development. In 2006, B73 lacked ears approximately 30% of the time. Regardless of year or infestation status, SDG15 and SDG19 had the lowest mean yield, which was below 20 g grain per plant. Poor agronomic performance of SDG15 and SDG19 is likely related to longer physiological maturation times (70–74 DTA; table 3), and thus poor pollination.

There was a significant effect of year on maize yield (Year,  $df_{1,87}$ ,  $F = 1.10$ ,  $P = 0.30$ ; Year  $\times$  Line,  $df_{7,87}$ ,  $F = 2.76$ ,  $P = 0.01$ ; Year  $\times$  Infestation,  $df_{1,87}$ ,  $F = 2.02$ ,  $P = 0.16$ ; Year  $\times$  Line  $\times$  Infestation,  $df_{7,87}$ ,  $F = 0.68$ ,  $P = 0.69$ ), and so data from each year were analysed separately. In 2005, maize line significantly influenced yield (tables 3 and 6), which was driven by low yields of SDG20, although Tukey's HSD post-hoc comparisons among lines were not significant. In 2005, the effects of rootworm infestation on yield were not consistent between maize lines, with three inbreds (SDG6, SDG9, SDG12) not exhibiting yield losses after rootworm infestation, thus resulting in a non-significant P-value for infestation effects. In 2005, infestation status did not impact the effect of maize line on yield, leading to a non-significant Line  $\times$  Infestation interaction.

Similar to 2005, maize line had a significant impact on yield in 2006 (tables 3 and 6), with SDG20 having some of the lowest mean yields. In contrast to the previous year, infestation status had a significant negative impact on yield, even though two inbreds (SDG6 and SDG12) did not exhibit yield losses after infestation with rootworms. As in 2005, the Line  $\times$  Infestation interaction was not significant.

#### Plant and primary ear node height

Plant height was significantly lower in 2005 than 2006 (Year,  $df_{1,89}$ ,  $F = 85.56$ ,  $P < 0.0001$ ), and although the interaction between year and maize line was not significant (Year  $\times$  Line,  $df_{11,89}$ ,  $F = 1.01$ ,  $P = 0.44$ ), the interaction between all three variables was marginally significant (Year  $\times$  Line  $\times$  Infestation,  $df_{12,89}$ ,  $F = 1.74$ ,  $P = 0.07$ ), and thus data from 2005 and 2006 were analysed separately. Maize line significantly impacted plant height in both years (tables 4 and 6). In check plots, the two public inbreds used in the recurrent selection breeding program had the lowest (W64A) and highest (B73) mean plant heights, therefore it is not surprising that the experimental inbreds had a range of plant heights. In 2005,

Maize line <sup>1</sup>	Plant ht. (m) <sup>2</sup> 2005		Plant ht. (m) <sup>2</sup> 2006	
	C	WCR	C	WCR
SDG11	2.08 ± 0.04 b	1.98 ± 0.08 abc	2.31 ± 0.04 abc	2.10 ± 0.15 bc
SDG20	2.01 ± 0.07 b	1.78 ± 0.09 c	2.21 ± 0.05 bc	2.07 ± 0.12 c
SDG10	2.08 ± <sup>3</sup> ab	2.02 ± 0.07 abc	2.32 ± 0.03 abc	2.49 ± 0.06 abc
SDG6	2.29 ± 0.01 ab	2.18 ± 0.06 ab	2.38 ± 0.15 abc	2.36 ± 0.14 abc
SDG12	2.36 ± 0.06 ab	2.11 ± 0.05 abc	2.66 ± 0.06 a	2.67 ± 0.16 ab
W64A	1.98 ± 0.01 b	1.84 ± 0.08 bc	2.09 ± 0.02 c	2.11 ± 0.07 c
SDG19	2.24 ± 0.08 ab	2.01 ± 0.05 abc	2.42 ± 0.10 abc	2.36 ± 0.02 abc
SDG17	2.33 ± 0.03 ab	2.04 ± 0.06 abc	2.27 ± 0.05 abc	2.36 ± 0.03 abc
SDG15	2.16 ± 0.16 ab	1.94 ± 0.09 abc	2.31 ± 0.10 abc	2.26 ± 0.05 abc
B73	2.54 ± 0.13 a	2.24 ± 0.06 a	2.55 ± 0.02 ab	2.77 ± 0.06 a
SDG9	2.31 ± 0.13 ab	2.32 ± 0.02 a	2.52 ± 0.12 abc	2.54 ± 0.13 abc
SDG7	2.27 ± 0.09 ab	2.14 ± 0.10 ab	2.45 ± 0.13 abc	2.22 ± 0.11 bc
All lines	2.23 ± 0.04	2.05 ± 0.03	2.37 ± 0.03	2.36 ± 0.03

Within each column means ± standard error of the mean followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>1</sup>W64A and B73 are susceptible public inbreds.

<sup>2</sup>C = non-infested control plants; WCR = plants infested with western corn rootworm eggs.

<sup>3</sup>No standard error because plants only germinated in one replicate.

infestation with rootworm eggs had a negative impact on plant height, while in 2006 a significant impact of infestation was not observed.

There was a significant effect of year on primary ear node height (Year,  $df_{1,89}$ ,  $F = 4.92$ ,  $P = 0.03$ ; Year × Line,  $df_{11,89}$ ,  $F = 2.02$ ,  $P = 0.04$ ; Year × Line × Infestation,  $df_{12,89}$ ,  $F = 0.67$ ,  $P = 0.78$ ), and so data from each year were analysed separately. Maize line had a significant impact on height of the primary ear node in both years (tables 5 and 6). Similar to plant height, infestation with rootworm

eggs only had a negative impact on primary ear node height in 2005, and did not have a significant impact the following year.

## Discussion

Historically, the majority of maize germplasm exhibiting rootworm resistance has been in the form of tolerance (Wilson and Peters 1973; Owens et al. 1974; Branson 1986; Riedell and Evenson 1993; Prischmann et al. 2007), which means plants have lar-

**Table 4** Mean plant height for 10 experimental maize inbred lines and two public lines

Maize line <sup>1</sup>	Ear ht. (m) <sup>2</sup> 2005		Ear ht. (m) <sup>2</sup> 2006	
	C	WCR	C	WCR
SDG11	0.95 ± 0.01 a	0.93 ± 0.05 abc	0.94 ± 0.05 bcd	0.91 ± 0.02 ab
SDG20	0.75 ± 0.06 a	0.60 ± 0.04 d	1.07 ± 0.04 abcd	0.97 ± 0.11 ab
SDG10	1.04 ± <sup>3</sup> a	0.95 ± 0.05 ab	1.07 ± 0.07 abcd	1.07 ± 0.04 ab
SDG6	1.50 ± 0.50 a	1.02 ± 0.06 ab	1.23 ± 0.08 ab	1.24 ± 0.10 a
SDG12	1.05 ± 0.07 a	0.95 ± 0.05 ab	1.01 ± 0.05 abcd	1.08 ± 0.09 ab
W64A	0.81 ± 0.02 a	0.75 ± 0.04 bcd	0.85 ± 0.04 d	0.85 ± 0.07 b
SDG19	1.23 ± 0.05 a	1.10 ± 0.04 a	1.27 ± 0.10 a	1.17 ± 0.05 ab
SDG17	1.06 ± 0.04 a	0.97 ± 0.05 ab	0.93 ± 0.04 cd	0.97 ± 0.02 ab
SDG15	1.03 ± 0.11 a	0.91 ± 0.05 abcd	1.21 ± 0.03 abc	1.11 ± 0.02 ab
B73	1.20 ± 0.05 a	1.27 ± 0.21 a	1.14 ± 0.02 abc	1.18 ± 0.10 a
SDG9	1.20 ± 0.04 a	1.11 ± 0.04 a	1.17 ± 0.06 abc	1.10 ± 0.05 ab
SDG7	1.27 ± 0.17 a	0.98 ± 0.04 ab	1.17 ± 0.08 abc	1.06 ± 0.04 ab
All lines	1.09 ± 0.05	0.96 ± 0.03	1.09 ± 0.03	1.06 ± 0.03

Within each column means ± standard error of the mean followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>1</sup>W64A and B73 are susceptible public inbreds.

<sup>2</sup>C = non-infested control plants; WCR = plants infested with western corn rootworm eggs.

<sup>3</sup>No standard error because plants only germinated in one replicate.

**Table 5** Mean primary ear node height for 10 experimental maize inbred lines and two public lines

**Table 6** Statistical information for yield data from non-infested maize lines and maize lines infested with western corn rootworms

Effect	df	F-value	P-value
Yield 2005			
Line	7,40	2.35	0.04
Infestation	1,40	0.27	0.61
Line $\times$ Infestation	7,40	1.13	0.37
Yield 2006			
Line	7,44	4.23	0.001
Infestation	1,44	4.58	0.04
Line $\times$ Infestation	7,44	1.91	0.09
Plant Height 2005			
Line	11,43	7.96	<0.0001
Infestation	1,43	26.44	<0.0001
Line $\times$ Infestation	11,43	0.71	0.72
Plant Height 2006			
Line	11,44	7.61	<0.0001
Infestation	1,44	0.23	0.63
Line $\times$ Infestation	11,44	0.99	0.47
Ear Height 2005			
Line	11,43	5.91	<0.0001
Infestation	1,43	7.57	0.009
Line $\times$ Infestation	11,43	0.58	0.83
Ear Height 2006			
Line	11,44	7.70	<0.0001
Infestation	1,44	1.31	0.26
Line $\times$ Infestation	11,44	0.50	0.89

ger root systems that can withstand herbivore damage (Painter 1951). Maize plants that are tolerant to rootworm damage can produce high grain yields despite having similar root damage ratings to susceptible plants (Branson et al. 1982), a characteristic that is related to root size and compensatory root growth (Branson 1986; Spike and Tollefson 1989; Riedell and Evenson 1993). However, root growth that occurs after rootworm damage can also negatively impact maize yield, especially when adequate moisture is present (Gray and Steffey 1998). Irrigating plants growing in hot, dry conditions can ameliorate yield losses due to rootworm damage (Riedell et al. 1992).

Although Assabgui et al. (1995) found that hydroxamic acids produced by maize roots are related to rootworm antibiosis, only a few studies have documented non-preference or antibiosis of maize lines to rootworms in the field (Branson et al. 1983; Hibbard et al. 1999), which is typically assessed using root damage ratings (Branson et al. 1981, 1983; Branson 1986). Non-preferred plants are not eaten or used for oviposition as frequently as preferred plants, while plants exhibiting antibiosis have negative effects on pest life history parameters or performance (Painter 1951).

In our study, experimental maize inbreds derived from *Tripsacum dactyloides*, a species resistant to corn rootworms (Branson 1971; Moellenbeck et al. 1995), had mean root damage ratings that ranged from 2.4 to 4.5. Of the *Tripsacum*-introgressed experimental maize inbreds, SDG11 and SDG20 consistently had some of the lowest mean root damage ratings, which were significantly lower than that of B73. This may indicate that these *Tripsacum*-introgressed lines are non-preferred by rootworms or have some measure of antibiosis. The root damage ratings of SDG11 and SDG20 were comparable to those of some experimental synthetic maize populations derived from cultivated maize tested in similar field trials, which also showed a range of susceptibility to rootworm damage (Prischmann et al. 2007).

Branson and Guss (1972) investigated the rootworm resistance properties of two sterile intergeneric  $F_1$  hybrids. The first hybrid was a cross between *Z. mays* (Sokota 250) and *T. dactyloides*, while the second was a cross between *T. dactyloides* and *Z. mays* (unknown variety). They compared the per cent of western corn rootworm larvae surviving to adulthood on each of the intergeneric hybrids versus a *Z. mays* hybrid. Larval survival was similar between the *Z. mays*  $\times$  *T. dactyloides* hybrid (25%) and the *Z. mays* line (20%). In contrast, no larvae completed development on the *T. dactyloides*  $\times$  *Z. mays* hybrid (0%). They concluded that rootworm resistance was inherited via the cytoplasm (i.e. the female parent), or that in the *Z. mays*  $\times$  *T. dactyloides* hybrid the genes responsible for rootworm resistance were on the genome that was not transferred during crossing.

In our study, although all experimental lines had some root damage, SDG11 and SDG20 had root damage ratings below 2.5. SDG11 had *T. dactyloides* as the male parent in the bridging cross with *Z. diploperennis*. SDG20 was derived from a three-way cross in which two of the parents had *T. dactyloides* as the female parent. The experimental lines were derived from a recurrent selection breeding program (detailed in the methods) in which the lines were backcrossed to a corn female. Furthermore, four genes on four different linkage groups, which are derived from *T. dactyloides*, are involved in maize resistance to rootworms (Eubanks 2003, 2006). Although Eubanks (2003, 2006) screened mitochondrial genes in the molecular marker mapping experiments, the four genes associated with rootworm resistance are all nuclear genes. Therefore, our experimental evidence does not appear to support the hypothesis of Branson and Guss (1972) for cytoplasmic inheritance of rootworm resistance from



*T. dactyloides*. The variability in root damage ratings among experimental inbreds in this study is likely due to differences in the number and identity of the four genes each line carries. There may be opportunities to increase levels of resistance to rootworms if the genes causing resistance in the *Tripsacum*-introgressed maize germplasm are different from those in the synthetic populations originating from cultivated maize, especially if experimental lines have high yield potential.

Grain yield is an important component of maize breeding programs, and in both years, mean yield of most experimental inbreds equalled or exceeded that of the two public inbreds, indicating that these experimental lines may have potential to be parents in breeding programs. However, lower root damage ratings did not necessarily translate into higher mean yields. After infestation, lines with the lowest root damage ratings had similar yields to those with the highest root damage ratings, and SDG20, which had consistently low root damage ratings, often had the lowest mean yield of lines used in analyses. This parallels results from Spike and Tollefson (1989), who found that root damage ratings were not consistent indicators of maize yield. Because seeds were not treated with fungicides, problems with plant germination in experimental inbreds may be due to mould, although most lines had germination and ear development rates similar to those of the public inbreds B73 and W64, and poor germination was not a factor in other field trials. Two lines, SDG15 and SDG19, had poor ear development and extremely low yield, which was likely because they matured later than the other lines (70–74 DTA), and were not successfully pollinated. Although DTA data were used as a covariate in yield analyses, due to the presence of pollen produced by buffer row plants the earliest maturing inbreds may have had a pollination advantage and therefore higher yields. However, the primary point of the study was to investigate effects of rootworm infestation on maize inbred performance, not to compare maize lines *per se*.

While the majority of lines had reduced yield after rootworm infestation, four lines (SDG6, SDG9, SDG10, and SDG12) did not exhibit yield losses after infestation with rootworms in one or both sample years, which is likely related to the stimulation of root growth and proliferation due to rootworm feeding (Riedell and Reese 1999), and indicates these lines may be tolerant to rootworm damage. SDG6 and SDG12 did not have yield loss in both years and their root damage ratings ranged from 3.1 to 3.5. This may be important for maize breeders to consider, as this

level of root damage in these genetic backgrounds may not have any negative impact on grain yield.

Thus, although *Tripsacum dactyloides* is resistant to corn rootworms via non-preference and/or antibiosis (Branson 1971; Moellenbeck et al. 1995; Eubanks 2001), *Tripsacum*-introgressed maize inbreds varied from being moderately resistant (SDG11 and SDG20) to susceptible to rootworms, while others appeared to be tolerant (SDG6 and SDG12). However, because emergence of adult rootworms was not monitored, it is possible that inbreds with low root damage ratings did not possess non-preference or antibiosis attributes, but were a highly nutritious food source that enhanced larval development with little root damage (Moeser and Hibbard 2005). It is also unclear what the underlying mechanism is that contributed to yield increases in tolerant inbreds.

One limitation of this study is that experimental germplasm was only evaluated in one location. Relationships between root damage, root size, and maize yield is often variable, and can be affected by several factors, including soil moisture, growing year, tillage, and location (Riedell et al. 1991, 1992; Gray and Steffey 1998). Data from multiple environments is needed before the rootworm resistance and agronomic potential of these inbreds is fully known, in addition to evaluating the performance of promising inbreds in testcross combinations. SDG LLC is currently conducting additional field tests in North Carolina, Iowa, Indiana, and in winter nurseries in Florida and Chile to assess the combining ability and yield potential of hybrids developed from experimental and commercial inbreds.

*Tripsacum*-introgressed maize germplasm also has drought tolerance and the ability to withstand aluminium toxicity in acidic soils (Eubanks 2006). Maize lines with genes responsible for adaptations to adverse growing conditions, along with genes conferring resistance and tolerance to rootworms could be useful in developing improved maize hybrids. In summary, some of the experimental inbreds we evaluated showed potential for further development in breeding programs. Further testing is needed to fully evaluate the potential of *Tripsacum* introgression to develop resistant or tolerant maize hybrids for use in rootworm control programs, particularly as refuge partners for transgenic maize and non-GMO markets.

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